Robust Summaries of All Data

ENVIRONMENTAL FATE – BIODEGRADATION	
Test Substance	
Chemical Name	Spent pulping liquor
CAS #	66071-92-9
Method	
Method/Guideline followed	Testing was conducted according to OECD Test Method 302 B, *"Zahn Wellens Test"*
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	2002
Contact time	28 days
Inoculum	Activated sludge from Haddington Sewage Treatment Works.
Test conditions	A preliminary test was conducted to investigate ready biodegradability potential of spent pulping liquor over 10 days following OECD (301B). This study indicated that the test material was unlikely to pass the ready biodegradability test requirement, with only 29% biodegradation during this time. Prior to initiation of the main test, the solubility of the test material in the mineral medium was investigated in order to establish an appropriate addition rate. This was found to be 193 mg/l based on dissolved organic carbon (DOC) from an addition rate of 200 mg/l. This was regarded as soluble for the purposes of the test. Test Setup: Mineral medium was prepared by adding 25 ml of a potassium phosphate solution and 25 ml each of three other solutions (magnesium sulfate, calcium chloride, ferric chloride) to make a total volume of 25 liters in demineralized water. Six bioreactor flasks were prepared: two each for controls and the test item and one each for reference (aniline 400 mg/l) and toxicity control. The test item was introduced to the reactor vessels at 200 mg/l equivalent to 185 mg of spent pulping liquor/2liter vessel, based on percentage carbon content. Each bioreactor had a total volume of 2000 ml. The control bioreactors each contained 185 ml of sludge and 1315 ml of mineral medium. The reference bioreactor contained 185 ml of sludge, 125 ml of the aniline stock solution and 1190 ml of mineral medium. The test bioreactors each contained 185 ml sludge, the appropriate weight of test item and 1215 ml of mineral medium. The toxicity control bioreactor contained 185 ml sludge, the appropriate weight of test item, 125 ml aniline stock and 1090 ml of mineral medium. The test was conducted over a 28 day period. DOC measurements were conducted on duplicate samples 3 h after test initiation and on days 14 and 28. Sampling frequency: Samples were collected for analysis on days 14 and 28.

	Controls: Yes. Method of calculating results: The weight of CO_2 evolved was calculated from the titre. The actual titre for each batch of $Ba(OH)_2$ prepared was used as the background titre. The mean titre for the test, reference and control vessels were calculated from the following equation: Weight CO_2 produced (mg)=1.1x(background titre-ml HCL titrated) The net CO_2 production was then calculated by subtracting the control mean CO_2 production from the test and reference material CO_2 production values. The percentage biodegradation was calculated by comparing actual CO_2 evolved in test and reference vessels with the theoretical CO_2 evolution.
Results	
Degradation % after time	Spent pulping liquor reached 71.6% degradation by Day 14 and 49.5% by Day 28. Based on the limited data, it is suspected that the values achieved by Day 14 resulted from adsorption to the sludge. Values on Day 28 are taken as more representative as solids content is largely depleted by the end of the study. The toxicity control reached 91.2% degradation by Day 14 and 88.1% by Day 28. As spent pulping liquor is a complex mixture with a proportion not entering into solution, these results can also be expressed based on carbon content of the initial test item added (200 mg organic carbon). On this basis, the degree of total material biodegradation is estimated to be 39% biodegradation.
Conclusions	Spent pulping liquor was not found to be readily biodegradable. However, a substantial level of inherent biodegradation was observed based on DOC depletion. Spent pulping liquor attained a level of 49.5% biodegradation by Day 28. This was equivalent to 39% biodegradation of the whole test material based on the initial DOC addition rate (200 mg DOC/l).
Data Quality	Reliable without restrictions– Klimisch Code 1a
Reference	Kelly, C.R. 2002. Spent Pulping Liquor. An Investigation of the Biodegradability of Spent Pulping Liquor. Inveresk Research, Tranent, Scotland.

ECOTOXICITY – ACUTE TOXICITY TO FISH	
Test substance	
Chemical Name	Spent pulping liquor
CAS #	66071-92-9
Method	
Method/Guideline followed	OECD Test Method 203, *"Testing of Chemicals, Fish Acute*

	Toxicity Test" and following procedures in OECD (2000) Guidance Document No. 23 on Testing Difficult Substances.
Year	2002
GLP (Y/N)	Y
System of testing	Fathead minnows (*Pimephales promelas)* under static conditions.
Concentration	0, 1, 10, 100 and 1000 mg/l
Results	The 96 hr LL_{50} was > 1000 mg/l the highest loading rate tested. The No Observed Effect Loading Rate ($NOEL_r$) was 1000 mg/l.
Detailed Summary	Spent pulping liquor was tested in fathead minnows under static conditions to determine the acute toxicity. Water accommodated fractions (WAF) were prepared by adding appropriate weights of spent pulping liquor to a stirring medium in glass vessels which were sealed to avoid loss of volatile fractions. Using magnetic stirrers, the stirring speed was adjusted to give a stirring vortex 5-10% of the water column. After a stirring period of approximately 48 hr. the test solutions were allowed to settle for ca hour. Since there was no evidence of precipitation or any undissolved material present, the entire WAF was adjusted to a pH of 8.0 The test organisms were exposed to these individually prepared WAF. This procedure was adopted to maximize the solubility of the test item under specific test exposure conditions. A control medium without the addition of the test item was stirred and treated in an identical way as the treated media. The effects of both filtering and adjusting pH (at 1000 mg/l) were investigated in a range finding test using loading rates of 0, 1, 10, 100 and 1000 mg/l. Because no mortality or other effects were observed in the range finding tests, a definitive-limit test was conducted at the maximum loading rate of 1000 mg/l. The 96 hr LL_{50} was > 1000 mg/l, the highest loading rate tested. The No Observed Effect Loading Rate ($NOEL_r$) was 1000 mg/l.
Data Quality	Valid without restriction – Klimisch Code 1a
Reference	Kelly, C. 2002. Spent Pulping Liquor CAS No. 66071-92-9 Determination of Acute Toxicity (LL_{50}) to Fathead Minnows (96 h, Static). Inveresk Research, Tranent, Scotland.

ECOTOXICITY – ACUTE TOXICITY TO DAPHNIA

Test substance

Chemical Name	Spent pulping liquor
CAS #	66071-92-9

Method

Method/Guideline followed	OECD Test Method 202, Part 1 *"Testing of Chemicals, Daphnia sp. Acute Immobilization Test"* and following procedures in OECD (2000) Guidance Document No. 23 on Testing Difficult Substances.
Year	2002
GLP (Y/N)	Y
System of testing	*Daphnia magna* (water fleas) under static conditions.

Concentration	0, 1, 10, 100 and 1000 mg/l
Results	The 48 hr EL_{50} was > 1000 mg/l the highest loading rate tested. The No Observed Effect Loading Rate ($NOEL_r$) was 1000 mg/l.
Detailed Summary	Spent pulping liquor was tested in daphnia under static conditions to determine the acute toxicity. Water accommodated fractions (WAF) were prepared by adding appropriate weights of spent pulping liquor to a stirring medium in glass vessels which were sealed to avoid loss of volatile fractions. Using magnetic stirrers, the stirring speed was adjusted to give a stirring vortex 5-10% of the water column. After a stirring period of approximately 48 hr. the test solutions were allowed to settle for ca hour. Since there was no evidence of precipitation or any undissolved material present, the entire WAF was adjusted to a pH of 8.0 The test organisms were exposed to these individually prepared WAF. This procedure was adopted to maximize the solubility of the test item under specific test exposure conditions. A control medium without the addition of the test item was stirred and extracted in an identical way as the treated media. The effects of both filtering and adjusting pH (at 1000 mg/l) were investigated in a range finding test using loading rates of 0, 1, 10, 100 and 1000 mg/l. Because no mortality or other effects were observed in the range finding tests, a definitive-limit test was conducted at the maximum loading rate of 1000 mg/l. The 48 hr EL_{50} was > 1000 mg/l, the highest loading rate tested. The No Observed Effect Loading Rate ($NOEL_r$) was 1000 mg/l.
Data Quality	Valid without restriction – Klimisch Code 1a
Reference	Kelly, C. 2002. Spent Pulping Liquor CAS No. 66071-92-9 Determination of Acute Toxicity (EL_{50}) to Daphnia (48 h, Static). Inveresk Research, Tranent, Scotland.

ECOTOXICITY – ALGA, GROWTH INHIBITION

Test substance

Chemical Name	Spent pulping liquor
CAS #	66071-92-9

Method

Method/Guideline followed	OECD Test Method 201, *"Testing of Chemicals, Alga, Growrh Inhibition Test"* and following procedures in OECD (2000) Guidance Document No. 23 on Testing Difficult Substances.
Year	2002
GLP (Y/N)	Y
System of testing	Green alga (*Selenastrum capriconutum)* growth inhibition.
Concentration	0, 125, 250, 500 and 1000 mg/l
Results	The 72 hr E_rC_{50} (Average specific growth rate) was estimated as 142.98 mg/l; the 72 h E_bC_{50} (AUC) was estimated as 312.44 mg/l with a corresponding No Observed Effect Loading Rate ($NOEL_r$) of 5 mg/l for AUC and Average Specific Growth Rate.
Detailed Summary	Spent pulping liquor was tested in alga to determine the median effective loading (EL_{50}) for growth inhibition. Water accommodated fractions (WAF) were prepared by adding

	appropriate weights of test substance to algal growth medium in glass vessels. Using magnetic stirrers, the stirring speed was adjusted to give a stirring vortex 5-10% of the water column. After a stirring period of approximately 48 hr. the test solutions were allowed to settle for ca hour. Since there was no evidence of precipitation or any undissolved material present, the entire WAF was adjusted to a pH of 8.0 The algae were exposed to these individually prepared WAF. This procedure was adopted to maximize the solubility of the test item under specific test exposure conditions. A control medium without the addition of the test item was stirred and extracted in an identical way as the treated media. The effects of both filtering and adjusting pH (at 1000 mg/l) were investigated in a range finding test using loading rates of 0, 1, 10, 100 and 1000 mg/l. In the range finding test there was a 100% inhibition of growth at 1000 mg/l, 34% at 100 mg/l, 29.9% at 10 mg/l and 16.7% at 1 mg/l. Based on the results of the range-finding test a definitive test was conducted at loading rates of 0, 1, 5, 10, 25, 125, 625, and 1000 mg/l. Algal cell concentrations were recorded after 0, 24, 48, and 72 h during the definitive test. The average specific growth rate was measured for each replicate flask during the experimental period, using daily cell counts. Growth curves were calculated for each test concentration and the area under each curve (AUC) determined. The results were determined based on average specific growth rates ($.day^{-1}$) and areas under growth curves ($cells.hr.ml^{-1}$) The 72 hr E_rC_{50} (Average specific growth rate) was estimated as 142.98 mg/l; the 72 h E_bC_{50} (AUC) was estimated as 312.44 mg/l with a corresponding No Observed Effect Loading Rate ($NOEL_r$) of 5 mg/l for AUC and Average Specific Growth Rate.
Data Quality	Valid without restriction – Klimisch Code 1a
Reference	Kelly, C. 2002. Spent Pulping Liquor, CAS No. 66071-92-9 Alga, Growth Inhibition Test (72 h, EL_{50}). Inveresk Research, Tranent, Scotland.

IN VITRO GENETIC TOXICITY	
Test substance	
Chemical Name	Spent pulping liquor
CAS #	66071-92-9
Method	
Method/Guideline followed	Test conducted according to OECD Test Method 471, *"Bacterial Reverse Mutation Test"*
Year	2001
GLP (Y/N)	Y
System of testing	*S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2*uvr*A

Concentration	0, 17, 50, 167, 500, 1667, and 5000 µg/plate
Metabolic activation	With and without addition of S-9 fraction from Aroclor 1254-treated Sprague-Dawley rats.
Results	Non-mutagenic
Detailed Summary	Spent pulping liquor was tested against *S. typhimurium* strains TA98, TA100, TA1535, and TA1538 and *E. coli* WP2*uvr*A for mutagenic activity. The test article was tested at concentrations of 0, 17, 50, 167, 500, 1667, and 5000 µg/plate with and without metabolic activation. Positive controls not requiring metabolic activation included sodium azide, 9-aminoacridine and 2-nitrofluorene; the positive controls requiring metabolic activation were 2-aminoanthracene and N-ethyl-N-nitro-N-nitrosoquanidine. No increases in mutation frequency were reported at any concentration of spent pulping liquor with or without metabolic activation. Spent pulping liquor was not mutagenic in this assay.
Data Quality	Valid without restriction – Klimisch Code 1a
Reference	Stevenson, F.M. 2001. Spent Pulping Liquor Testing for Mutagenic Activity with *Salmonalla typhimurium* TA 1535, TA 1537, TA 98 and TA 100 and *Escherichia coli* WP2uvrA. Inversk Research, Tranent, Scotland.

IN VITRO GENETIC TOXICITY	
Test substance	
Chemical Name	Spent pulping liquor
CAS #	66071-92-9
Method	
Method/Guideline followed	OECD Test Method 473, *"Chromosomal Aberration Assay with Chinese Hamster Ovary Cells in vitro."*
Year	2001
GLP (Y/N)	Y
System of testing	Chinese Hamster Ovary (CHO) cells *in vitro*
Concentration	With S9 mix: 625, 1250, 2500 and 5000 ug/ml Without S9 mix: 1250, 2500 and 5000 ug/ml
Metabolic activation	With and without addition of S-9 fraction from Aroclor 1254-treated adult male Fisher rats.
Results	Clastogenic with S9 mix at 2500 ug/ml and without S9 mix at 5000 ug/ml; both concentrations were overtly toxic to the cells.
Detailed Summary	Spent pulping liquor was tested in Chinese hamster ovary (CHO) cells for clastogenic activity both with and with metabolic activation with rat liver S9 mix. The test article was tested with metabolic activation with S9 mix at concentrations of 625, 1250, 2500 and 5000 ug/ml and without metabolic activation with S9 mix at concentrations of 1250, 2500 and 5000 ug/ml. The positive controls requiring and not requiring metabolic activation were cyclophosphamide (CPH) and methanesulphonate (MMS), respectively. Treatments with test item or controls were performed on duplicate cell cultures. Two slides per culture up to 50 metaphase cells per slide were examined. A dose level was considered to be toxic if the cell count was reduced to less than 50% of the mean vehicle control values or if consistent evidence of changes to cell morphology was observed. In both the presence and absence of S9 mix, positive levels of structural aberrations were observed. In the presence of S9 mix, this response was observed in the cultures treated with 2500 ug/ml and in the absence of S9 mix, in the cultures treated with 5000 ug/ml. Both of these concentrations were judged overtly toxic to the cultures. Therefore, tall oil fatty acid was a clastogen at toxic concentrations.
Data Quality	Valid without restriction – Klimisch Code 1a
Reference	Murie, E. 2001. Spent Pulping Liquor Chromosomal Aberration Assay with Chinese Hamster Ovary Cells in vitro (Complying with EC (Annex V) and OECD 473 Guidelines). Inveresk Research, Tranent, Scotland.